To Die or Not to Die

An Overview of Apoptosis and Its Role in Disease

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The death of cells in tissues of humans and other multicellular organisms is neither always abnormal nor always detrimental. Although necrosis ensues at the sites of massive cellular injury, most cells in the body die through a more subtle, noninflammatory, energy-dependent form of cell death called *apoptosis*. The number of cells in tissues is determined by the homeostatic balance between proliferation of new cells and death of senescent cells; the rates of proliferation and apoptosis vary widely from tissue to tissue. Recent research into the molecular mechanisms of apoptosis has revealed that apoptosis is a genetically programmed process that can become deranged when the components of the cellular apoptotic machinery are mutated or present in inappropriate quantities. Dysregulation of apoptosis is associated with the pathogenesis of a wide array of diseases: cancer, neurodegeneration, autoimmunity, heart disease, and other disorders. Products of genes involved in the regulation and execution of apoptosis are potentially excellent targets for diagnosis and therapeutic intervention in disease processes, and they offer renewed hope for cures and treatments for a wide array of maladies.

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PAINSTAKING RESEARCH in histology, genetics, and molecular biology during the past 25 years has revealed that virtually all animal cells are armed with the genetic machinery to commit suicide. Under normal physiological circumstances, damaged or senescent cells sacrifice themselves for the greater good of the whole organism through a genetically programmed type of cell death termed *apoptosis*. As is true of other physiological systems, cell number in the body is subject to homeostasis: the number of cells in a multicellular organism equals the rate of cell proliferation minus the rate of cell elimination. Although it has long been known that excessive proliferation of cells leads to neoplasia and that insufficient proliferation can lead to developmental agenesis of body structures, only recently has it become apparent that aberrations in the regulation of genetically programmed cell death likewise cause disease and deformity.

This review begins by defining the 2 principal patterns of cell death (necrosis and apoptosis), subsequently describes well-understood genes of apoptosis in the nematode worm *Caenorhabditis elegans*, and then discusses the current understanding of molecular apoptosis regulation and execution in human cells. These first sections provide scientific background for understanding the increasingly apparent roles of apoptosis in disease and the potential for apoptosis-based clinical diagnosis, prognosis, and therapy in the future.

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TYPES OF CELL DEATH

Necrosis

Cells of multicellular organisms generally die in 1 of 2 well-characterized ways, depending on the context and cause of death (Table). Necrosis occurs in acute, nonphysiological injury (eg, in the center of infarcted tissue in an ischemic stroke or at the site of toxin action). Necrotic cells swell and lyse, releasing their cytoplasmic and nuclear contents into the intercellular milieu, thus sparking inflammation. Although important in acute injury and certain severe inflammatory responses, necrosis is not the mechanism by which cells normally die. Until the early 1970s, necrosis was the only clearly identified type of cell death, thus making cell death seem a nonphysiological and detrimental event.

Apoptosis

In 1972, Kerr et al[1] published a seminal article describing the novel physiological process of apoptosis (derived from the Greek word for "falling off"). Cells undergoing apoptotic "cellular suicide" rapidly shrink and lose their normal intercellular contacts and subsequently exhibit dense chromatin condensation, nuclear fragmentation, cytoplasmic blebbing, and cellular fragmentation into small apoptotic bodies. These apoptotic bodies are quickly phagocytosed and digested by neighboring cells or macrophages.[1-5] As no cytosolic contents are released into the intercellular medium during apoptosis, inflammation is not triggered. The discovery of apoptosis was a remarkable feat of histology: in a slide of normal tissue consisting of millions of cells, only a handful will show structural changes characteristic of apoptosis at any given time, as the sequence of morphological changes in apoptosis can be completed in less than an hour.

The stereotypical sequence of histological events in apoptosis led to the idea that apoptosis is a form of programmed cell death directed in part or whole by the apoptotic cell itself. Apoptotic cell death is an integral part of development and of homeostasis in adult tissue. The developing mammalian central nervous system (CNS), for example, is especially prone to apoptosis, as many more neurons are generated than survive into adulthood, either the result of limiting amounts of growth and survival factors (the neurotrophic hypothesis) or the inability to make functional connections (the activity hypothesis). In adulthood, apoptosis is greatly reduced in the mammalian CNS, but it continues actively in other tissues. For example, mature granulocytes exist in the peripheral circulation for only 1 to 2 days before undergoing apoptosis and clearance. Apoptosis can also be actively induced in animal cells by a diverse array of other triggers, which range from ionizing radiation to hyperthermia to viral infections to immune reactions.[4-6]

Cardinal Features of Apoptosis and Necrosis*

Features	Necrosis	Apoptosis
Stimuli	Toxins, severe hypoxia, massive insult, and conditions of ATP depletion	Physiologic and pathological conditions without ATP depletion
Energy requirement	None	ATP-dependent
Histology	Cellular swelling, disruption of organelles, death of patches of tissue	Chromatin condensation, apoptotic bodies, death of single isolated cells
DNA breakdown pattern	Randomly sized fragments	Ladder of fragments in internucleosomal multiples of 185 base pairs
Plasma membrane	Lysed	Intact, blebbed, with molecular alterations
Phagocytosis of dead cells	Immigrant phagocytes	Neighboring cells
Tissue reaction	Inflammation	No inflammation

*ATP indicates adenosine 5'-triphosphate.

THE WORM'S TALE: A GENETIC MODEL OF CELL DEATH

The genetics and molecular mechanisms of apoptosis were first characterized in the late 1980s and early 1990s in studies of the nematode worm *C elegans*. Programmed cell death during *C elegans* development is extremely precise and predictable: specific genes are activated to kill exactly 131 cells, leaving 959 in the adult worm.[7] Apoptosis in development can be thought of as a normal cell fate, like terminal differentiation into muscle or nerve.

Studies of the worm revealed that apoptosis consists of 4 sequential steps: (1) commitment to death by extracellular or intracellular triggers, (2) cell killing (execution) by activation of intracellular proteases, (3) engulfment of the cell corpse by other cells, and (4) degradation of the cell corpse within the lysosomes of phagocytic cells (Figure 1, A).[8] These stages, and the genes that govern them (termed *ced* genes in *C elegans*, for cell death abnormal), are remarkably conserved throughout animal evolution, from worm to human (Figure 1, B).[8-10]

In *C elegans*, the protein products of the genes *ced-3* and *ced-4* are required for the execution of apoptosis,[11] and the product of the *ced-9* gene prevents apoptosis by inhibiting activation of *ced-3* and *ced-4* (Figure 1, A).[12] CED-3, a cysteine aspartyl protease (caspase), is necessary for apoptosis: when activated, it cleaves a variety of cellular proteins, inactivating some and activating others. These death substrates of the CED-3 caspase include DNA repair enzymes such as polyadenosine diphosphate ribose polymerase, components of the nuclear membrane, and endonucleases responsible for cleaving the apoptotic cell's DNA. Activation of caspases in a cell's cytoplasm is directly associated with the morphological changes characteristic of apoptosis. The activation of caspases, whose inactive precursors (procaspases) are present in all cells, causes breakdown of the normal barriers between cellular compartments, thus wreaking havoc within the cell but leaving the plasma membrane virtually intact.

CED-4 acts upstream of CED-3: CED-4 receives a death commitment signal and subsequently binds to pro–CED-3, causing it to release active CED-3. CED-9, a multifunctional protein that is localized to the outer membranes of mitochondria and other intracellular membranes, binds to CED-4 and prevents its activation of pro–CED-3, possibly by anchoring CED-4 away from cytoplasmic pro–CED-3.[13-15] Therefore, CED-3 and CED-4 induce apoptosis, and CED-9 protects against apoptosis.

MOLECULAR MECHANISMS OF APOPTOSIS IN HUMANS

The *C elegans* model is relevant to higher animals because of its evolutionary conservation. Several central mediators of apoptosis in mammals have similar molecular shapes and signaling roles as CED-3, CED-4, and CED-9 (Figure 1 and Figure 2). Humans and other mammals are more complex than worms; so, too, is the regulation of apoptosis in mammalian cells, which involves many more signaling molecules, particularly at the early stages of commitment to apoptosis.

Commitment to Die

Some mammalian death-commitment pathways are ubiquitous: for example, all normal cells respond to radiation-induced DNA damage by signaling cell cycle arrest, which can be followed by resumption of cycling once the damage is repaired or commitment to apoptosis if the damage is too severe. Other death-commitment signaling pathways are present only in specific cell types and at certain times. The relative abundances of different signaling molecules determine the cell's apoptotic threshold by determining the relative speed, strength, and likelihood of activating apoptotic and antiapoptotic pathways. The advantage of a variety of cell death signals is clear: if there were only 1 signal for cell death, all cells exposed to that signal would die. With multiple different cell death triggers, specific subsets of cells can be deleted without collaterally eliminating other types of cells adjacent to them.

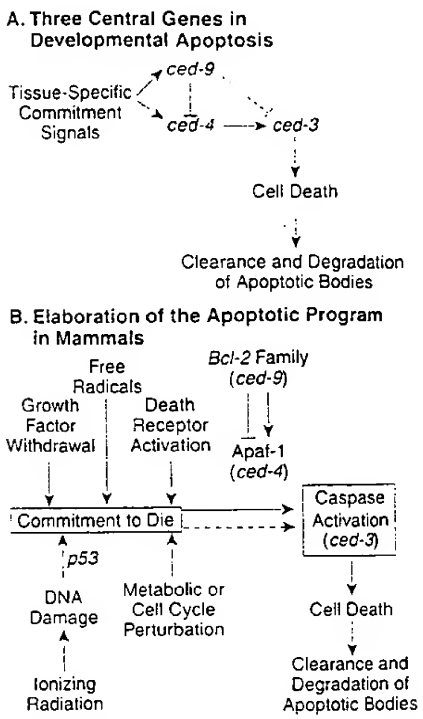

Figure 1.—The apoptotic program is conserved in evolution. A, Tissue-specific signals activate *ced-4*, which activates *ced-3*, leading to cell death. If activated, *ced-9* can inhibit apoptosis by inhibiting *ced-4*'s activation of *ced-3* (and possibly by directly inhibiting *ced-3*), as indicated by dashed arrow. B, A wide array of factors commit a mammalian cell to die, but the downstream apoptotic machinery is conserved (worm genes homologous to mammalian apoptotic genes are shown in parentheses). Arrows indicate positive interactions; blunted arrows, negative interactions.

Extracellular Death Ligands and Receptors

Commitment to death can be specifically triggered by addition of a death-promoting ligand to the extracellular medium around a cell in culture or in the body. When extracellular death ligands bind to cell-surface death receptors, the intracellular portion of the receptor changes shape and is able to bind to cytoplasmic adaptor proteins (Figure 2).[16-18] Receptor-adaptor complexes subsequently bind to downstream adaptor proteins that can activate a variety of caspases—the proteases homologous to CED-3 that act as the executioner in mammals.[19-21]

The immune system provides excellent examples of how death ligands and receptors actively kill specific infected or otherwise dangerous cells of the body. As shown in Figure 3, A, when the T-cell

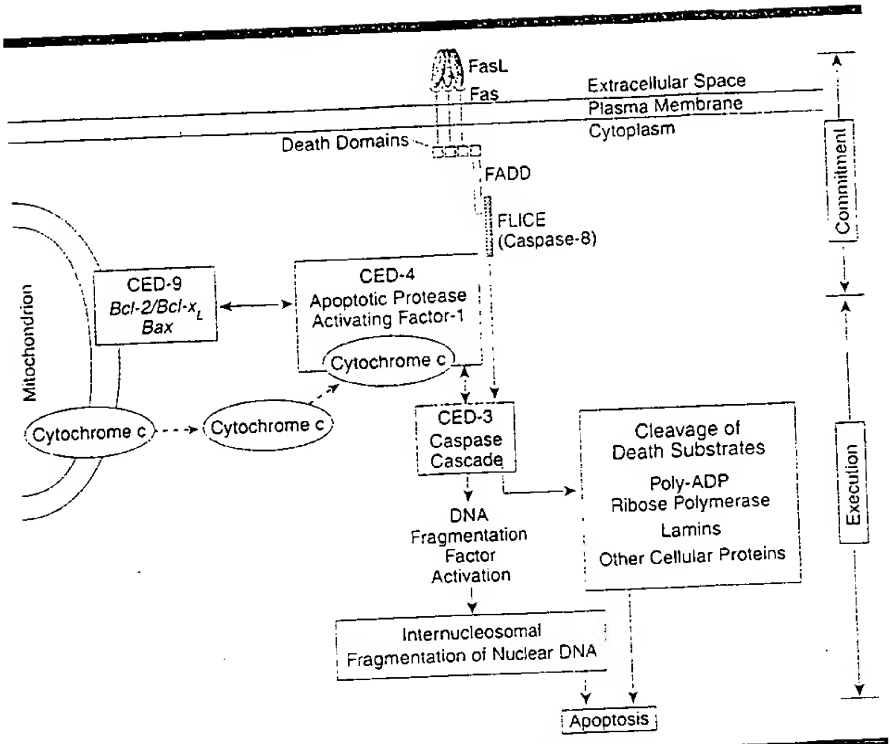

Figure 2.—Apoptotic signaling pathways. Commitment to death: death ligand FasL binds to death receptor Fas to initiate intracellular signaling; the adaptor protein FADD (Fas-associated death domain protein) binds Fas and also binds to caspase-8 (also known as FLICE, a FADD-like interleukin 1β–converting enzyme). Execution: activation of caspase-8 leads to the activation of other caspases; caspases cleave each other's inactive procaspase precursors into active caspases, amplifying the death signal; active caspases mediate execution by cleaving polyadenosine diphosphate ribose polymerase, nuclear lamins, and other cellular proteins and by activating DNA fragmentation factor. Modulation of apoptotic signaling: in some cell types, cytochrome c is released from mitochondria, allowing activation of apoptotic protease activating factor-1 (Apaf-1), also leading to caspase activation; *Bcl-2* family members may promote or suppress apoptosis by interacting with Apaf-1 and/or regulating mitochondrial permeability. Arrows do not necessarily imply direct interactions; homologous *Caenorhabditis elegans* gene products are shown in gray.

receptor on a cytotoxic T lymphocyte (T_c cell) binds to a foreign peptide (eg, a fragment of a viral protein) presented on the class I major histocompatibility complex of an antigen-presenting cell, it induces the expression of the protein Fas ligand (FasL) on the surface of the T_c cell. FasL then binds to Fas, a cell-surface receptor present on most cells of the body, and activated Fas initiates an intracellular apoptotic signaling pathway that kills the infected antigen-presenting cell. A similar mechanism also protects the body from T lymphocytes with defective T-cell receptors that inappropriately bind to self-antigens as if they were foreign antigens (Figure 3, B1).[22-25] As might be expected, failure to delete self-reactive T cells can lead directly to autoimmune disease.

Death by Neglect

Commitment to death can also be specifically triggered by removal of a death-inhibiting (or, survival-promoting) ligand. In this form of death by neglect, a cell commits to die because it lacks cytoplasmic signals from a cell-surface survival-factor receptor. This dearth of exogenous signals allows an endogenous default death program to play itself out.[24-27] For example, activated T lymphocytes are dependent on the soluble protein interleukin 2 (IL-2) for survival and undergo apoptosis if IL-2 is removed (Figure 3, B2). The same final execution processes are activated whether apoptosis is initiated by a death ligand or by lack of a survival factor.

Execution: Adaptors, Caspases, and Death Substrates

A variety of death commitment signals in mammalian cells converge to activate the central executioner: the caspase cascade (Figure 1, B). For example, after cell-surface death receptors such as Fas are activated, their cytoplasmic tails bind to adaptor proteins such as FADD (Fas-associated death domain protein) (Figure 2).[17] The Fas-FADD complex then binds to and activates caspase-8,[21] which initiates the lethal proteolytic cascade of apoptosis execution.

In contrast to the single CED-3 caspase in *C elegans*, at least 10 different caspases have been isolated from human cells. Activated caspases cleave each others' precursors into mature, active enzymes in a proteolytic cascade similar to complement activation or blood clotting.[23,28] As in *C elegans*, human death substrates include cytoplasmic and nuclear proteins involved in DNA repair and replication, RNA splicing, cytoskeletal structure, and cell division.[25,28] Once caspases are activated, the morphological changes of apoptosis ensue, and the killing process cannot be halted.

A Human CED-4 Homologue

As in *C elegans*, humans have a CED-4 homologue that induces apoptosis, called *apoptotic protease activating factor-1* (Apaf-1).[29] When the mitochondrial protein cytochrome c binds to Apaf-1, Apaf-1 is able to bind to and activate human caspase-3, initiating the caspase cascade (Figure 2).[30] Interestingly, Apaf-1 also has a binding site for adenosine 5′-triphosphate (ATP), which might explain why the ATP energy level in an injured cell may play a critical role in deciding whether the cell has sufficient energy to die by apoptosis or if instead it dies by energy-independent necrosis. Finally, Apaf-1 can also bind to antiapoptotic CED-9 homologues of the *Bcl-2* family, which may sequester Apaf-1 away from caspase-3, thus suppressing apoptosis.

The *Bcl-2* Family and the Uncertain Role of Mitochondria in Apoptosis

Named for its founding member, a gene isolated from a B-cell lymphoma that caused oncogenesis by suppressing apoptosis, the *Bcl-2* family of genes includes both apoptosis-promoting (eg, *Bax* and *Bad*) and apoptosis-inhibiting (eg, *Bcl-2* and *Bcl-x_L*) members.[31-33] The *Bcl-2* family members promote or inhibit apoptosis induced by certain triggers, such as growth factor withdrawal–induced apoptosis, but they do not always affect other kinds of death signaling, such as the Fas pathway in certain cell types.[29,27,34]

How exactly *Bcl-2* family members function to promote or inhibit apoptosis is uncertain. The leading theories are that antiapoptotic *Bcl-2*–like proteins inhibit caspase activation either by binding directly to Apaf-1, by preventing the release of cytochrome c and other contents from mitochondria, or both.[35,36] The *Bcl-2* family members are located in the outer membranes of mitochondria, can bind to each other in various pairwise combinations (eg, homodimers of *Bcl-2*: *Bcl-2* or heterodimers like *Bcl-2*:*Bax*), and form ion-conducting channels in the mitochondrial membrane. Antiapoptotic *Bcl-2* family members may form ionic pores that allow electrochemical homeostasis in cellular organelles; conversely, proapoptotic family members may interfere with channel formation.[37,38] Altered

mitochondrial membrane potential—often an early warning sign of apoptosis—may allow mitochondrial contents to leak into the cytoplasm, where they activate caspases.[29,31,32,39,40] Thus, *Bcl-2* family members act upstream of caspases: once the cascade is started, antiapoptotic *Bcl-2* family members cannot usually prevent cell death. A recent report also indicates that Bcl-2 itself may be a downstream death substrate of caspases, implying the existence of a more complex relationship between *Bcl-2* family members and caspases.[41]

Final Steps of Apoptosis: Engulfment and Degradation of Apoptotic Bodies

Engulfment and degradation of apoptotic bodies complete the process of programmed cell death; however, these processes are less well understood than commitment and execution. Changes in the makeup of cell surface proteins (eg, thrombospondin up-regulation) and lipids (eg, presentation of phosphatidylserine) allow apoptotic bodies to be recognized by other cells for phagocytosis. Phagocytosis of apoptotic corpses whose plasma membranes are intact is a key feature of apoptosis, as there is no leakage of proinflammatory cytosolic components. The final degradation of apoptotic bodies is poorly understood. Defects in this process in humans have been linked to some forms of systemic lupus erythematosus, as inappropriate processing of apoptotic bodies may allow formation of antinuclear antibodies.[42]

ROLES OF APOPTOSIS IN DISEASE

The survival of multicellular organisms requires a balance between cell proliferation and cell death. Abnormal regulation of apoptosis has been implicated in the onset and progression of an ever-broader range of diseases. Many disorders can be classified based on whether they are associated with too much or too little apoptosis.[6] During their pathogenesis, however, most apoptotic disorders feature too much apoptosis of one type of cell and too little elimination of another kind of cell. Thus, apoptotic disorders can be classified based on their primary dysfunction, which may later lead to other (and opposite) dysfunctions.

This review discusses only a few diseases associated with dysregulated apoptosis. Completely omitted are discussions of the roles of apoptosis in acute trauma, myocardial infarction, stroke, and infectious diseases (such as viral hepatitis and the acquired immunodeficiency syndrome). These and other topics are reviewed extensively elsewhere; MEDLINE lists hundreds of recent articles relating apoptosis to disease.

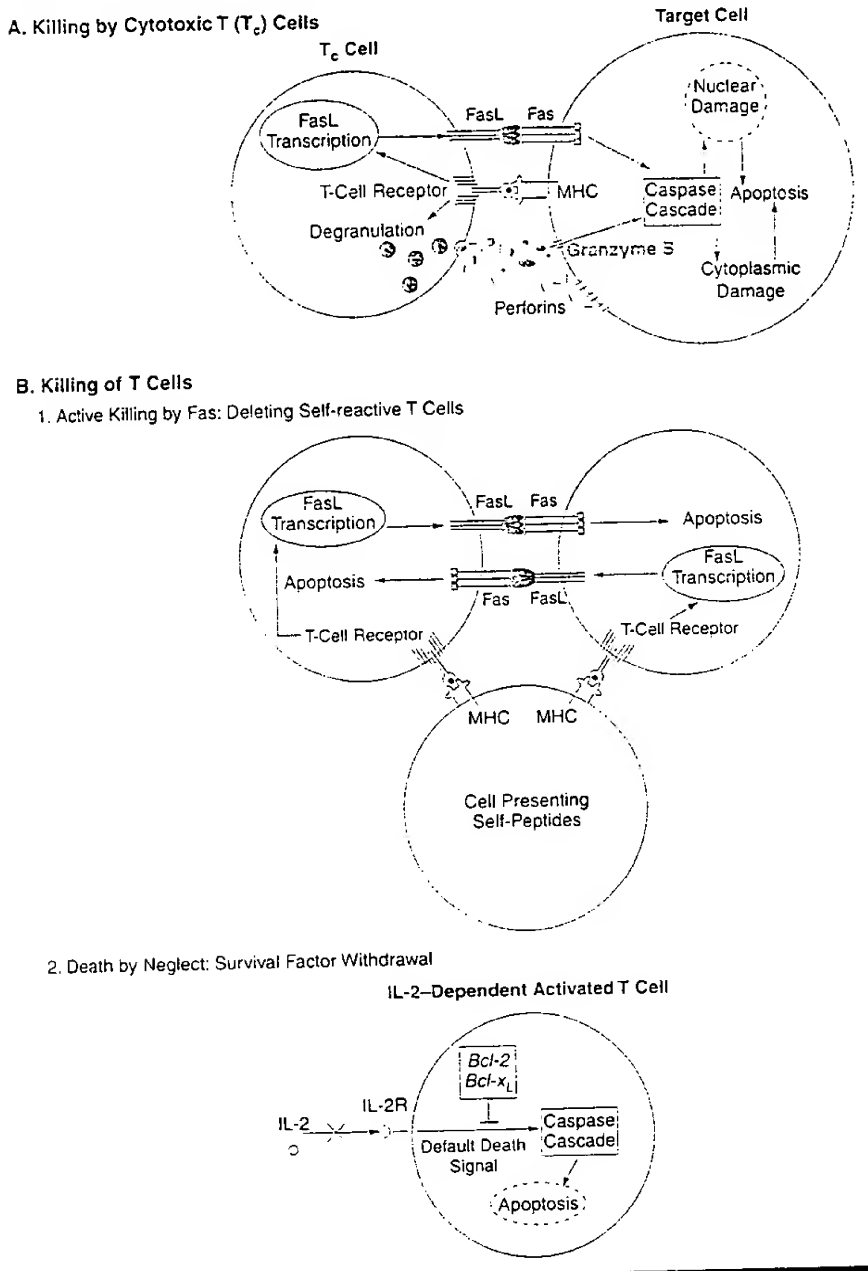

Figure 3.—Active and passive killing pathways. A, Cytotoxic T cells (T_c cells) kill in 3 ways: Fas ligand (FasL) on the T_c cell binds to Fas on the target cell, inducing apoptosis; T_c cells degranulate, releasing both granzyme B, which enters the target cell and directly activates caspases, and perforins, which can form pores in the target cell membrane, causing osmotic lysis. B, T cells can be killed actively or passively. (1) Peripheral self-tolerance: if the T-cell receptor of a T cell binds avidly to self-peptides presented on a self–MHC I (a class I major histocompatibility complex) on a peripheral cell, the T cell may be induced to express FasL, in addition to Fas, thus allowing self-reactive T cells to kill each other and prevent autoimmunity (B1 has been adapted, with permission, from Nagata[23]). (2) Death by survival factor withdrawal: if an activated T cell that expresses the interleukin 2 receptor (IL-2R) is deprived of interleukin 2 (IL-2), it will undergo apoptosis because of a default death signal that can be suppressed by antiapoptotic members of the *Bcl-2* family.

Primary Apoptosis Deficiencies

Graft Rejection.—Not all parts of the body are immunologically equivalent: certain sites and tissues are protected by immune privilege from inflammation and other collateral damage associated with vigorous immune responses.[43] Allografts and xenografts, usually destroyed by host responses, are able to thrive in sites of immune privilege such as the cornea and testis. One potential mechanism for mediating immune privilege relies on the Fas-FasL apoptosis pathway (Figure 2).[44] Whereas Fas is expressed constitutively (as in hepatocytes) or inducibly (as in lymphocytes) on a wide variety of cell types, only ac-

tivated T cells and somatic cells in immune privileged sites usually express FasL.[25,44] Lymphoid cells are able to infiltrate sites of immune privilege but are subsequently induced to undergo apoptosis when their Fas receptors bind FasL. Thus, rejection of nonprivileged grafts from nonprivileged sites can be paradoxically viewed through the Fas-FasL model as a primary underabundance of apoptosis (in the host's lymphoid cells): reactive T cells of the host are not killed by graft's cells (which do not express FasL). These infiltrating T cells may then go on to induce apoptosis in or otherwise kill cells of the graft, leading to a secondary elevation in apoptosis (in the graft's cells) during graft rejection. In a strict sense, however, graft rejection is not a pathological primary deficiency of apoptosis; rather, it is the normal physiological process of an intact immune system destroying foreign cells.

Autoimmune Diabetes.—As mentioned in the discussion of how T lymphocytes kill cells via Fas-FasL interactions, since elimination of self-reactive lymphocytes (peripheral self-tolerance) depends on apoptosis, all autoimmune disorders can be viewed as primary deficiencies of apoptosis. Observations about the roles of Fas and FasL have led to new perspectives on the pathogenesis and treatment of autoimmune diabetes mellitus.[45] As modeled in nonobese diabetic mice, disease progression is regulated at 2 checkpoints: nonreactive infiltration of the islets of Langerhans by leukocytes and initiation of active destruction of pancreatic β cells by T cells, a process that culminates in insulin-dependent diabetes.[45] Fas and FasL appear to act at the second checkpoint. Although islet allografts are usually rejected, this rejection can be prevented in some strains of mice by cotransplanting myoblasts engineered to express FasL.[46] Artificially expressing FasL does not always protect islets from diabetogenic T cells, however. For example, in another set of studies, constitutive expression of FasL in pancreatic β cells resulted in induction of Fas on β cells by T cells, thus making the β cells even more vulnerable to killing by T cells (or by each other, as they now expressed Fas and FasL).[47,48] In the development of autoimmune diabetes, the primary defect in apoptosis regulation is the failure of peripheral self-tolerance, which results in too little apoptotic deletion of self-reactive T cells. Secondary to the presence of these dangerous lymphocytes, cells of the pancreas are destroyed.

Local Self-reactive Disorders.—Dysregulation Fas and FasL expression following infections can cause cells of tissues to induce their neighbors to undergo apoptosis in a form of local autoimmunity. A recently proposed mechanism for Hashimoto thyroiditis is based on the Fas-FasL system.[49] Normal and Hashimoto thyroiditis thyrocytes express FasL and thus seem like immune-privileged cells. Stimulation of thyrocytes by IL-1β during an infection or other inflammation, for example, leads to expression of Fas along with FasL on thyrocytes. This unfortunate state of affairs may cause thyrocytes to kill each other, thus explaining the high rate of apoptosis in Hashimoto thyroiditis. Other recent experiments have shown that Fas-mediated hepatocyte death in hepatitis can be blocked by specific pharmacological inhibition of caspases.[50,51]

Lymphoproliferation and Autoimmunity.—Failure of peripheral self-tolerance of T lymphocytes—through deficiencies in either anergy or Fas-mediated apoptosis—may also underlie the pathogenesis of lymphoproliferative disorders.[23-25] If self-reactive lymphocytes become activated and are not removed or tolerized in a timely manner, they may enhance the likelihood of an autoimmune reaction. Mice with Fas or FasL gene mutations have autoimmune disorders and excessive lymphocyte levels. Fas is mutated in mice with the lymphoproliferation (lpr) mutation; FasL is defective in mice with the generalized lymphoproliferative disorder (gld) mutation. These strains of mice have been extremely useful in examining the roles of Fas and FasL in peripheral tolerance and autoimmunity.[22,23]

Dominant negative and recessive mutations in the *Fas* gene that prevent transduction of the apoptotic signal have been found recently in at least 12 pediatric patients with autoimmune lymphoproliferative syndromes (ALPS), including the Canale-Smith syndrome.[52-54] These patients manifest signs and symptoms that are reminiscent of those seen in mice lacking the *Fas* (lymphoproliferation [lpr] mutation): accumulation of nonmalignant immature T lymphocytes in secondary lymphoid organs (giving rise to marked adenopathy), polyclonal hypergammaglobulinemia, autoantibody production, thrombocytopenia, neutropenia, and glomerulonephritis. When *Fas* genes of 50 normal individuals were examined, no mutations in *Fas* were found[53]; this argues in favor of a causative role for *Fas* mutations in ALPS. Mutations and allelic differences in other genes affect the severity of phenotype in humans and mice with *Fas* mutations, perhaps indicating that mutations in different parts of the apoptotic pathways can alter the severity of any given *Fas* defect. Genetic analysis of patients' apoptosis genes may thus be diagnostically and prognostically useful.

Cancer.—Cancer is an aberrant net accumulation of atypical cells, which can arise from an excess of proliferation, an insufficiency of apoptosis, or a combination of the 2. Until recently, models of cancer pathogenesis and antineoplastic therapies focused on the role of proliferation in cancer. Thus, highly toxic chemical and radiation therapies that damage DNA and cytoskeletal elements involved in cell cycling and division were developed with the intention of targeting rapidly dividing tumor cells preferentially over slower-dividing normal body cells. Ideally, whereas normal body cells were thought to arrest their cell cycle and repair DNA and cytoskeletal damage, deranged cancer cells were thought to continue cycling and accumulating mutations until they died of gross genomic or metabolic dysfunction. The clinical observation that many slow-growing cancers are curable and many rapidly dividing cancers are refractory to treatment, however, demonstrates that proliferative models of cancer are inadequate.[55] Insight into apoptosis has opened a new dimension on understanding and treating cancer.

Apoptosis is of critical importance both to the pathogenesis of cancers and to their likelihood of resistance to conventional antineoplastic treatments. Mutations in the *p53* gene and its regulators (eg, *mdm2*) are extremely common, occuring in perhaps 55% to 70% of human cancers.[56] In response to DNA damage, the p53 protein induces cell cycle arrest and, in some circumstances, apoptosis. The p53-deficient cells are inefficient at DNA repair, as they do not arrest the cell cycle and properly activate DNA repair functions, yet they are generally more resistant to radiation therapy than cells containing functional p53 protein.[55] Loss of functional p53 correlates well with tumor aggressiveness in a variety of tumors, and people inheriting a defect in 1 of their 2 copies of the *p53* gene (Li-Fraumeni syndrome) develop cancers at a high rate.[55]

The protein p53 induces apoptosis by acting as a transcription factor, activating expression of numerous apoptosis-mediating genes. Of particular interest, several *p53*-induced genes encode proteins that regulate the redox state of cells.[57] A current model proposes that DNA damage causes the p53 protein to turn on genes whose products generate free radicals that, in turn, damage the cell's mitochondria, whose contents (such as cytochrome c) leak out into the cytoplasm and activate apoptotic caspases.[57]

The p53 protein can also induce apoptosis by up-regulating expression of *Bax*, a proapoptotic *Bcl-2* family member. Tu-

mors exhibit varying numbers of apoptotic cells; a high proportion of apoptotic cells correlates with slowed tumor growth. Conversely, mice that lack the genes for *Bax* develop fast-growing tumors that have 50% fewer apoptotic cells than similar tumors from mice with normal *Bax* genes.[58] *Bax* apparently also functions in a p53-independent pathway for colon carcinogenesis of the microsatellite mutator type.[59] Hence, there are likely several different possible pathways to apoptosis, even in cancer cells. As expected, when antiapoptotic members of the *Bcl-2* family are overexpressed, the likelihood of neoplasia is greatly enhanced: *Bcl-2* was first isolated from a B-cell follicular lymphoma and classified as a tumor suppressor before its role in apoptosis was discovered.[22]

The concept of immune privilege can help us understand why tumors evade the apoptosis-inducing effects of the immune system. Metastatic malignant melanoma cell lines have been found that express FasL, thus allowing the tumor cells to kill T lymphocytes that venture near.[60] The role of FasL in tumor immune escape is supported by the observation that FasL-expressing mouse melanoma cells grow more slowly in mice lacking Fas than in normal mice. In a study of 22 hepatocellular carcinomas from human patients, all the tumors had down-regulated their expression of Fas and undergone novel expression of FasL.[61] Thus, these tumor cells not only expressed a ligand to kill lymphocytes, but they also removed their own Fas receptors, rendering themselves impervious to that form of apoptosis induction.

In summary, mutations in genes that lead direcly or indirectly to reduced apoptosis are generally associated with poor prognosis in a variety of tumor types, since conventional chemotherapy and radiation therapy rely primarily on induction of apoptosis in cancer cells for therapeutic effect.[6,52] New cancer therapies that aim to induce apoptosis specifically in cancer cells are the source of much excitement and renewed hope for cures.

Primary Apoptosis Excesses

Neurodegenerative Disorders.—Gradual loss of specific types of neurons from different parts of the CNS characterizes the pathological progression of a variety of neurodegenerative disorders. As the CNS is a site of intense apoptosis during development (some estimate 50%-80% of CNS neurons die during development) and appears to depend on the expression of survival-promoting genes like *Bcl-x_L* for survival in adulthood, it may be expecially vulnerable to derangements of apoptotic pathways, particularly pathways involving calcium[62] and free-radical generation.[63] Apoptotic cell death and its attendant molecular mediators appear to play a role in many neurodegenerative disorders, including Alzheimer disease, Parkinson disease, spinal muscular atrophy, and amyotrophic lateral sclerosis.[6,38,64,65]

Mutations in the presenilin 2 gene have recently been associated with familial Alzheimer disease.[66] The presenilin 2 is hypothesized to function in an apoptosis pathway downstream of Fas. Presenilin 2's mouse homologue prevents the up-regulation of FasL on T lymphocytes, which normally occurs when the T cell is activated by binding to a foreign peptide.[66] In the PC12 neuronal cell line, overexpression of normal presenilin 2 leads to apoptosis; a mutant presenilin 2 isolated from patients with familial Alzheimer disease has an even greater ability to induce apoptosis.[67] One of the cardinal features of Alzheimer disease, formation of amyloid β plaques in the brain, also alters the apoptotic threshold of neurons. In primary cultures of human neurons, peptide fragments of amyloid β can down-regulate antiapoptotic *Bcl-2* and up-regulate proapoptotic *Bax* expression, thus making the neurons more prone to die, especially in response to oxidative stress.[68]

Parkinson disease is characterized by the degeneration of nigrostriatal dopaminergic neurons, which are thought to die by apoptosis and necrosis in response to oxidative damage.[69,70] Although predisposing genes for Parkinson disease have been more elusive than those for Alzheimer disease, the treatment for Parkinson disease has turned out to be intimately involved in the inhibition of apoptosis. The drug selegiline hydrochloride has been used historically as a treatment for Parkinson disease because of its ability to irreversibly inhibit monoamine oxidase B, thus enhancing dopamine signaling. The effect of selegiline on monoamine oxidase B may be of less importance than the recent discovery that it can specifically alter transcription of cellular death and survival genes, including superoxide dismutases, *Bcl-2* and *Bcl-x_L*, nitric oxide synthase, and nicotinamide adenine dinucleotide dehydrogenase.[71] Selegiline prevents the progressive reduction in mitochondrial membrane potential and thus may inhibit the release of proapoptotic substances from the mitochondria.

Heart Disease.—Several recent studies have investigated the extent of apoptosis and the levels of the molecular mediators of apoptosis in the failing human heart. In one study, 7 hearts explanted at the time of transplantation surgery were assayed for apoptosis.[72] All 4 hearts with idiopathic dilated cardiomyopathy (IDCM) and 1 with ischemic cardiomyopathy had apoptotic nuclei, as measured by specific labeling of broken DNA in an assay called *terminal deoxyuridine nucleotide end labeling* (TUNEL).[73] Additionally, the DNA from the IDCM hearts yielded an internucleosomal DNA laddering pattern characteristic of apoptosis when subjected to agarose gel electrophoresis. The other 2 hearts with ischemic cardiomyopathy did not show signs of apoptosis either on TUNEL or electrophoretic assay.

In a similar but larger study,[74] other investigators assayed for apoptosis the hearts from patients with IDCM, ischemic cardiomyopathy, and valvular heart disease. Control hearts averaged a mean (SD) of 10 (9) TUNEL-positive nuclei per million nuclei, IDCM averaged 2366 (2033) per million, ischemic cardiomyopathy averaged 2436 (1964) per million, and the 1 case of valvular heart disease had 1015 TUNEL-positive nuclei per million. Clearly, apoptosis is elevated (up to 232 times the control level) in failing hearts in this study, but the percentages of cells undergoing apoptosis are far less than those in the previous study. This discrepancy may reflect variability in the TUNEL assay, which theoretically labels apoptotic nuclei before they appear pyknotic (as DNA fragmentation begins before pyknotic changes). The specificity and sensitivity of TUNEL have not been determined; thus, no quantitative conclusions can be drawn from TUNEL results.[75] This study also examined levels of Bcl-2 and Bax proteins in the failing hearts: although Bax expression remained constant, Bcl-2 expression averaged 1.8 times the level in control hearts. Thus, in the failing heart, the normally antiapoptotic action of Bcl-2 is insufficient to overcome death-promoting stimuli (such as mechanical stretch, inflammatory cytokines, or reactive oxygen species) that may be present in the failing heart.

A third study[76] also finds evidence of apoptosis in heart failure resulting from arrhythmogenic right ventricular dysplasia, a dangerous disorder characterized by progressive replacement of myocardium by adipose and fibrotic tissue. This study found high levels of caspase-3 expression, thus providing a mechanism of cell death. These early, correlative studies have revealed that apoptosis is increased in hearts failing for a variety of reasons. Whether increased apoptosis is the primary cause of cell death or is secondary to an as yet unknown process remains to be seen.

APOPTOSIS-BASED DIAGNOSIS, PROGNOSIS, AND THERAPY

Understanding the physiological process of apoptosis at the molecular level

not only affords inights into disease pathogenesis but also opens new avenues for developing diagnostic, prognostic, and therapeutic tools. TUNEL and other assays that can detect early stages of apoptotic processes before morphological changes are apparent in histological sections will allow easier diagnosis of conditions involving apoptosis. Considering that only the last hour or so of the apoptotic process is morphologically observable, a much higher proportion of diseases may involve inappropriate levels of apoptosis than currently described. The causes of overabundant or underabundant apoptosis will be discernible as apoptotic signaling pathways are more fully elucidated and assays for these novel signaling molecules are developed. A biopsy specimen from a case of idiopathic liver degeneration, for example, might be screened for new diagnostic parameters: deficiencies of growth or survival factors and antiapoptotic intracellular proteins, elevations in proapoptotic molecules, inappropriate activation of caspases, and oxidative mitochondrial damage. Assaying for mutations in apoptosis-related genes has already begun to refine prognosis in a variety of cancers; future assays not only for mutations in genes but also for aberrant levels of gene expression in cancers will allow better selection of therapies to which the tumor should be specifically susceptible.

Insight into the mechanisms of apoptosis has already led to a surge in research into novel therapies for degenerative, neoplastic, and autoimmune disorders. Rational drug design opens the door to highly specific therapies with fewer adverse effects. Potential therapies fall into 3 broad categories: (1) gene therapy (eg, replacement of *p53*), (2) injectible molecules targeted at the upstream modulators of apoptosis (eg, growth factors or soluble FasL), and (3) small molecule pharmaceuticals designed to regulate expression of apoptosis-related genes (eg, *Bcl-2*). For apoptosis-based therapy to be feasible, therapeutic molecules must be delivered to and active only in specific target cells: indiscriminate inhibition of apoptosis could lead to widespread hyperplasia, and inappropriate promotion of apoptosis might lead to undesirable tissue degeneration. A further caveat is that saving a cell from death is not necessarily the same thing as preserving its function. Preventing the death of neurons in the substantia nigra of patients with Parkinson disease by injection of neurotrophic factors or other antiapoptotic molecules does not necessarily mean that they will remain fully differentiated and continue to secrete dopamine.

Considering the complexity of apoptosis signaling, combinatorial therapies that target multiple cell death molecules for activation or inactivation may offer the best hope of therapeutically modulating apoptosis. If one wanted to inhibit caspase activation, one would currently have to use several inhibitors: although multiple caspases upstream of caspase-3 can be inhibited by viral proteins such as cowpox CrmA and baculovirus p35, synthetic tripeptides and tetrapeptides inhibit caspase-3 and other caspases specifically.[23,77] Understanding the molecular apoptosis pathways is also beginning to allow the development of novel nonpharmaceutical therapies. For example, an adenovirus that is only able to replicate in and kill p53-deficient cells is in phase 1 clinical trials as a possible antitumor agent that should kill only p53-deficient tumor cells, leaving normal cells unaffected.[78]

CONCLUSIONS

Molecular apoptosis research is moving extremely rapidly; however, a few themes are durable. First, apoptosis is a highly conserved mechanism among multicellular animals. Second, there are multiple paths of commitment to death and multiple components of the central executioner. Third, the apoptotic threshold of a cell fundamentally depends on the ratios and relative abundances of different positive and negative regulators. Fourth, apoptosis plays a primary or secondary role in many pathological states and diseases. Finally, apoptosis represents an exciting new physiological process that may yield more definitive diagnosis, more accurate prognosis, and novel therapies for age-old ills.

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